SYNOPSIS OF PROPOSED RESEARCH

FOR

DOCTORAL DEGREE IN BIOINFORMATICS

"Comprehensive screening and validation of small molecule inhibitors for inflammatory markers"

Submitted to

DEPARTMENT OF BIOINFORMATICS
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Synopsis

"Comprehensive screening and validation of small molecule inhibitors for inflammatory markers"

1. Introduction:

Inflammation is a response to tissue injury or infection, and it is characterized in its acute phase by the increase in vascular permeability by plasma extravasations, resulting in an accumulation of fluid, leucocytes and mediators to the inflammatory site [1]. Inflammatory diseases are the vast array of disorders and condition caused by inflammation that includes allergy, asthma, autoimmune diseases, coeliac disease, glomerulonephritis, hepatitis, inflammatory bowel disease, reperfusion injury and transplant rejection [2]. During most of these events, many cytokines and enzymes expression were modulated and such cytokines and enzymes are TNF-α, IL-6, IL-1β, 5-LOX, COX-2, and PDE4B are crucial targets for numerous inflammatory diseases.

TNF-α

Tumor necrosis factor alpha (TNF-α) is among one of the pleiotropic cytokine, mainly secreted by monocytes and macrophages, TNF-α has been shown to play a pivotal role in orchestrating the cytokine cascade in many inflammatory diseases and it is known to be "master-regulator" of inflammatory cytokine production, notably it has been proposed as a therapeutic target for a number of diseases [3].

IL-6

IL-6, discovered in the 1980s as a lymphocyte-derived signal for B-cell maturation, functions as both a pro- and anti-inflammatory cytokine and has been implicated in a variety of inflammatory and autoimmune disorders [4]. IL-6 is a 184–amino acid cytokine produced by both immune cells, including macrophages, B cells, and T cells, and non-immune cells, such as endothelial cells and fibroblasts, in response to homeostatic disturbances such as infection and injury. IL-6 is a critical cytokine for the differentiation and growth of T and B cells [5].

IL-1β

IL-1β is a 153-amino acid cytokine produced by activated blood monocytes and tissue macrophages such as microglia that affects most cell types and promotes inflammation by

indirectly promoting lymphocyte function and activating macrophages [6]. IL-1β up-regulates endothelial cell expression of the adhesion molecules, which bind to leukocyte integrins and promote infiltration of inflammatory cells into tissue.

5-LOX

5-LOX catalyses the oxidation of arachidonic acid to 5-hydroperoxyeicosatetratenoic acid (5-HPETE), which is then converted into unstable epoxide leukotriene A4 (LTA4). Then, LTA4 undergoes either (i) hydrolysis by LTA4 hydrolase or (ii) conjugation with glutathione to form leukotriene B4 (LTB4) and cysteinyl leukotrienes (cysLTs) respectively. CysLTs consist of LTC4, LTD4, and LTE4 [7,8]. CysLTs, are known as the 'slow reacting substances of anaphylaxis', which induce bronchoconstriction and neutrophil extravasation and also play a role in mucus secretion [9,10]. LTB4 is the most powerful pro-inflammatory chemotactic agent compared to other leukotrienes [11,8]. It stimulates monocytes and T cell to produce TNF-α, IL-6 and MCP-1 that involve in inflammation [11]. Due this it can be considered as therapeutic target for inflammatory disease.

COX-2

Cyclooxygenase (COX) is also known as prostaglandin-endoperoxide synthase, exist in two isoform, COX-1 as well as COX-2 and is the key enzyme that converts arachidonic acid to prostaglandin, which impart crucial role in signaling and inflammation [12]. COX-1 is expressed in many tissues and prostaglandins (PGs) produced by COX-1 mediate the "housekeeping" functions like platelet aggregation, renal blood flow regulation and cytoprotection of gastric mucosa. Contrarily, COX2 is not detectable under normal circumstances but is activated by stress or other stimuli, such as inflammatory cytokines, growth factors, mitogenic, or hormonal stimuli

PDE4

Phosphodiesterase-4 (PDE4), mainly present in immune cells, epithelial cells, and brain cells, manifests as an intracellular non-receptor enzyme that modulates inflammation and epithelial integrity. Inhibition of PDE4 is predicted to have diverse effects via the elevation of the level of cyclic adenosine monophosphate (cAMP) and the subsequent regulation of a wide array of genes and proteins. It has been identified that PDE4 is a promising therapeutic target for the treatment of diverse pulmonary, dermatological, and severe neurological diseases. Over the past decades, numerous PDE4 inhibitors have been designed and synthesized, among which roflumilast,

apremilast, and crisaborole were approved for the treatment of inflammatory airway diseases, psoriatic arthritis, and atopic dermatitis, respectively [13].

The anti-inflammatory biology approach is mainly based on monoclonal antibody (mAbs), the fusion protein and biosimilars are prevalent since decade, to mention few mAbs; infliximab, golimumab, adalimumab, and cetrolizumab-pego, crelizumab, rilonacept,etc are presently used for the treatment of inflammatory diseases. Therefore, there was a continuous hunt for a new molecule which is having limited or no side effects since from many years. Small molecule inhibitors are likely to be cheaper to produce as compared with mAbs or Ig-Fc based fusion protein [14]. Few small molecule inhibitors have come to light, due to the side effects the usage is less. Currently, as due to advancement of modelling and simulation techniques, has created large opportunities and reliability to develop the drug molecule based on *in-silico* method.

In this study, the small molecule inhibitors for inflammatory markers will be designed based on *In-silico* method (using Schrodinger drug discovery platform), by predicting Pharmacophore and Pharmacokinetics properties of ligand and also evaluating the binding interaction between protein and ligand molecule. After successful designing of the small molecule, *Insilico* validation studies will be studied using various computational tools.

2. Objectives:

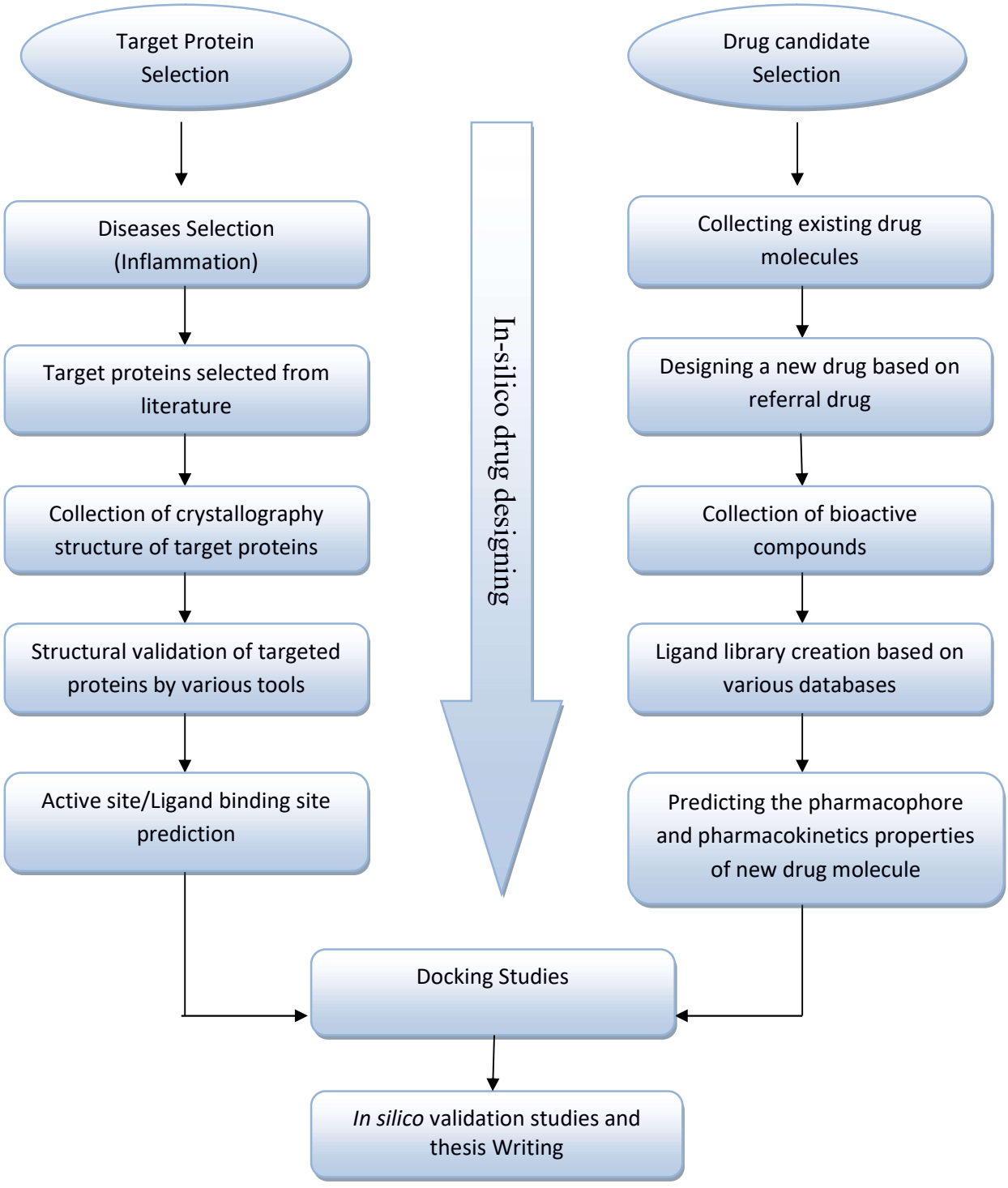
- ❖ Structural characterization of pro-inflammatory markers.
- ❖ Designing of small molecule inhibitors based on referral drug.
- ❖ Comprehensive screening of phytochemical based small molecule inhibitors for pro-inflammatory markers.
- ❖ Comprehensive screening of small molecule inhibitors from Drugbank and Asinex databases for pro-inflammatory markers.
- ❖ *Insilico* validation of screened compounds.

3. Significance of study:

The study helps to address the therapeutic potential of small molecule inhibitors and explore the opportunity to treat inflammatory and its related diseases/disorders.

4. Methodology and Workflow for the proposed research work:

3D structure of inflammatory markers are fetched from PDB database. Structure analysis and validation will be done by SAVS tool. Active site inflammatory markers of will be found by PDBsum/site map. Further ligand preparation will be studied using ACD/ChemSketch software to add all chemical compositions. The pharmacophore and pharmacokinetics properties of new ligand molecule will be studied using Hyperchem 7.5 Professional and Schrodinger suit packages. The resultant molecule of ligand and protein are docked by using glide and autodock tool [15-17]. Insilico validation done by molecular dynamic simulation.


Target Protein Selection
Diseases Selection (Inflammation)
Target proteins selected from literature
Collection of crystallography structure of target proteins
Structural validation of targeted proteins by various tools
Active site/Ligand binding site prediction
In-silico drug designing
Drug candidate Selection
Collecting existing drug molecules
Designing a new drug based on referral drug
Collection of bioactive compounds
Ligand library creation based on various databases
Predicting the pharmacophore and pharmacokinetics properties of new drug molecule
Docking Studies
In silico validation studies and thesis Writing

5. Time frame for the proposed research work:

	Work plan	I Year	II Year	III Year	IV Year
1	Literature work				
2	Course work				
3	Synopsis preparation				
4	Protein targets selection and structural charecteization				
5	Collecting and studying the existing drug molecules structures and designing new molecule.				
6	Bioactive compound screening				
9	Small molecule ligand library creation				
10	Pharmacophore and pharmacokinetics analysis of ligand molecule and manuscript preparation				
11	Docking studies				
12	*Insilico* validation studies				
14	Manuscript preparation and publication				
15	Thesis writing				

Note: Every year will participate in minimum of one workshop and a conference related to proposed research work. Approximately, the proposed research work will take the period of four years to complete and it may be extended for one year based on the progress if required.

6. References

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